Contact Lens and Anterior Eye 41 (2018) 277–281

Contents lists available at ScienceDirect

Contact Lens and Anterior Eye

journal homepage: www.elsevier.com/locate/clae

Antimicrobial efficacy of a novel povidone iodine contact lens disinfection system

Katsuhide Yamasaki, Fumio Saito, Ritsue Ota, Simon Kilvington*

Ophtecs Corporation. 5-2-4 Minatojima-Minami-Machi, Chuo-ku, Kobe, Hyogo, 650-0047, Japan

ARTICLE INFO

Keywords:
Acanthamoeba
Povidone iodine
Contact lens
Biocidal
Biofilm

ABSTRACT

Purpose: Contact lens (CL) wear is a risk factor for the acquisition of microbial keratitis. Accordingly, compliance to manufacturers' recommended hygiene and disinfection procedures are vital to safe (CL) use. In this study we evaluated a novel povidone-iodine (PI) (CL) disinfection system (cleadew, Ophtecs Corporation, Japan) against a range of bacterial, fungal and *Acanthamoeba*.

Methods: Antimicrobial assays were conducted according to ISO 14729 using the recommended strains of bacteria and fungi, with and without the presence of organic soil. Regrowth of bacteria and fungi in the disinfection system was also examined. The activity on biofilms formed from *Stenotrophomonas maltophilia* and *Achromobacter* sp. was evaluated. Efficacy against *A. castellanii* trophozoites and cysts was also investigated.

Results: The PI system gave > 4 $\log_{10}$ kill of all bacteria and fungi following the manufacturer's recommended disinfection and cleaning time of 4 h, with or without the presence of organic soil. No regrowth of organisms was found after 14 days in the neutralized solution. In the biofilm studies the system resulted in at least a 7 $\log_{10}$ reduction in viability of bacteria. For *Acanthamoeba*, > 3 $\log_{10}$ kill of trophozoites and 1.1–2.8 $\log_{10}$ kill for the cyst stage was obtained.

Conclusions: The PI system effective against a variety of pathogenic microorganisms under a range of test conditions. Strict compliance to recommended CL hygiene procedures is essential for safe CL wear. The use of care systems such as PI, with broad spectrum antimicrobial activity, may aid in the prevention of potentially sight threatening microbial keratitis.

1. Introduction

It has been estimated that there are some 140 million contact lens wearers worldwide [1]. With the exception of daily wear disposable lenses, all require some form of chemical disinfection for their safe use [2]. Failure to ensure good lens hygiene practices can result in sight threatening infection of the cornea (keratitis) by bacteria, fungi and the free-living amoeba *Acanthamoeba*. Microbial keratitis is a rare but significant risk among soft contact lens wearers with a reported incidence of 4 cases per 10,000 users per year [1]. In keratitis due to the free-living amoebae *Acanthamoeba*, almost 90% of cases occur in contact lens wearers and the reported incidence varies from 1 to 2 cases per million in the USA or 17–20 in the UK [3].

Typically, contact lens disinfection is achieved using multipurpose disinfectant solutions (MPS) or hydrogen peroxide (H_2O_2). The majority of MPS are based on the preservative polyhexamethylene biguanide (PHMB) but other formulations use quaternary ammonium compound polyquaternium-1 (PQ-1) alone or in combination with the amidoamine myristamidopropyl dimethylamine (MAPD), PHMB or alexidine [4]. With H_2O_2 disinfectants, the 3% solution is neutralized during disinfection through the use of a platinum disc inside the case or the separate addition of a catalase tablet [5].

Most recently has seen the introduction of an alternative contact lens disinfection method based on the chemical povidone iodine (PI). The system comprises of a contact lens storage case, diluent and a tablet containing the PI at 500 ppm, giving 50 ppm active iodine (cleadew, Ophtecs Corporation, Japan). The tablet is composed of two components: the first contains the PI and an inner core of ascorbic acid and the enzyme protease. Lenses are placed into the storage case which is filled with a suitable volume of diluent (8 ml). The tablet is then added which immediately begins to dissolve, releasing the PI from the outer layer and turning the solution a dark brown colour. After 5 min, the ascorbic acid is then released which neutralizes the PI turning the solution clear to indicate disinfection is complete which occurs after approximately 20 min. Finally, the protease layer is released which acts to remove proteins from the lens surface. To ensure complete disinfection and

* Corresponding author.
E-mail address: simon_kilvington@ophtecs.co.jp (S. Kilvington).

https://doi.org/10.1016/j.clae.2017.12.001
Received 5 December 2016; Received in revised form 4 November 2017; Accepted 1 December 2017

Table 1
Microbial Efficacy of cleadew System against Bacteria, Fungi and *Acanthamoeba*.

Microbiology test	Log10 kill (4 h)							
	P. aeruginosa (ATCC 9027)	*S. marcescens* (ATCC 13880)	*S. aureus* (ATCC 6538)	*C. albicans* (ATCC 10231)	*F. solani* (ATCC 36031)	*A. castellanii* (ATCC 50370 trophozoites)	*A. castellanii* (ATCC 50370 cysts: NNA)	*A. castellanii* (ATCC 50370 cysts: Neff)
Biocidal ("stand alone")	> 4	> 4	> 4	> 4	> 4	≧5	1.1	2.8
Biocidal ("stand alone") +0.03% "organic soil"	< 1	< 1	< 1	< 1	< 1	NT[a]	NT	NT
Regimen	< 1	< 1	< 1	< 1	< 1	< 1	< 1	NT
Regimen + 0.03% "organic soli"	< 1	< 1	< 1	< 1	< 1	< 1	< 1	NT
Biocidal: tap water (Leicester) as diluent	> 4	> 4	> 4	> 4	> 4	> 4	NT	NT
Biocidal: Japanese tap water (soft water) as diluent	> 4.5	> 4.5	> 4.3	> 4.5	> 4.3	> 4.5	NT	NT
Biocidal: Japanese drinking water (hard water: cortex) as diluent	> 4.5	> 4.9	> 4.5	> 4.4	> 4.2	> 4.5	NT	NT

[a] NT = not tested.

removal of protein, the manufacturer's recommended soaking time is 4 h. The diluent contains 40 ppm hydrogen peroxide which reacts with I^- formed in the neutralisation stage to generate additional I_2 to further enhance the biocidal activity of the system. Hydrogen peroxide at this concentration is not toxic to the cornea, as 100 ppm is required before ocular sensitivity occurs [5,6].

In this study we determined the antimicrobial efficacy of the PI system against a range of microbes, including those commonly associated with keratitis. The system showed good activity against all bacteria, fungi and *Acanthamoeba* according to international standards, including ISO 14729 and FDA 510(k) and has prompted this report [7,8].

2. Methods

For the ISO:14729 microbiological analysis, the following organisms were studied: *Pseudomonas aeruginosa* (ATCC 9027), *Serratia marcescens* (ATCC 13880), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231) and *Fusarium solani* (ATCC 36031) conidia. Testing was conducted in accordance with the international standards ISO 14729 and FDA 510(k). For the bacteria and fungi both stand-alone (biocidal) and regimen assays were performed according to ISO 14729 [7]. Briefly, in the stand-alone procedure the test solutions or Dulbeccos's Phosphate Buffered Saline (DPBS), negative control, were challenged with 1×10^6/ml organism and the number of survivors determined by culture viability after 0 and 4 h using the WASP Spiral Plater and ProtoCOL colony counter system or pour-plate methods [9]. At each time point the test samples were first diluted 1:10 into neutralizing broth consisting of sodium thiosulfate (0.6%), plant catalase (500 U/ml) and Tween 80 (0.05%) in DPBS, before determining the number of surviving organisms. The experiments were also repeated in the presence of organic soil consisting of a final concentration of approximately 3000 heat-killed (100 °C for 10 min) *Saccharomyces cerevisiae* (ATCC 9763) in 0.03% heat-inactivated foetal bovine serum (from stock of approximately 3×10^6 cells in 100% serum).

In some experiments, the challenged storage cases were left at 25 °C for 14 days before re-culturing to determine whether bacterial or fungal regrowth occurred. In others, the storage cases were sampled after 20 min as this is the time by which neutralization of the PI will have occurred.

In the regimen assay, three sets of the following lenses were tested per organism: Acuvue® Advance® (galafilcon A, Vistakon), O_2 OPTIX® (lotrafilcon B, CIBA Vision) and PureVision® (balafilcon A, Bausch & Lomb). Although not required by the protocol, 0.03% organic soil (see above) was also included in the organism challenge inoculum. Briefly, three lenses of each test type were inoculated with approximately 2×10^6 organisms and left to adhere for 5 min at 25 °C in a humidified chamber. Each lens was then subjected to the manufacturer's recommended lens care protocol. At the end of the regimen procedure, each test lens and soaking solution was placed into neutraliser followed by filtration and culture of the lens and membrane to determine the presence of surviving organisms. For the *Acanthamoeba,* the lenses were observed microscopically for any remaining, adhered trophozoites or cysts.

To test the efficacy of the system against biofilm formed bacteria, *S. maltophilia (*ALC01), *Achromobacter* sp. (S4) were suspended in 8 ml 0.01% trypticase soy broth and inoculated into 6 well microtiter plates (Nunc) for incubation, in air, at 32 °C for 24 h. Following rinsing of the wells three times DPBS, the wells were subjected to disinfection using the PI system with DPBS acting as a control. After 4 h, the biofilm was disrupted by sonication (50% amplitude for 3 s × 3) and the number of surviving bacteria determined as described above. Control wells were treated with DPBS only.

Acanthamoeba castellanii (ATCC 50370) biocidal efficacy was conducted as described previously using both the trophozoite and cyst form of the organism [10]. Cysts were produced using starvation of trophozoites plated on 2.5% non-nutrient agar (NNA) or Neff's chemically defined encystment medium [10,11]. The efficacy of the system against trophozoites using bathroom tap water (Leicester) and tap water and drinking water (Japan) was also tested to represent non-compliant use. Here, the tap water, in place of the system diluent, was challenged with 1×10^5 trophozoites and the povidone iodine tablet added. After 4 h the solution was centrifuged at 500 × *g* for 5 min, the supernatant discarded and the remaining pellet inoculated on a 1.5% NNA plate seeded with *Escherichia coli* (ATCC 8739) or into proteose-peptone-yeast extract (PYG) medium. The plates or culture flasks were incubated in air at 32 °C and inspected microscopically for 7 days for the presence of trophozoite growth and replication. Replacing the tablet diluent with tap or drinking water was also tested against the ISO:14729 panel of bacteria and fungi using the stand-alone protocol, as described above.

In addition, the capacity of the neutralized PI system to induce trophozoite encystment was investigated during incubation at 32 °C for 24 h, as described previously [12]. Neff's encystment medium containing 0.5% propylene glycol was used as a positive control.

The biocidal efficacy of the diluent only against *S. maltophilia* (ALC01), *Achromobacter* sp. (S4), *P. aeruginosa* (W9298), *P. aeruginosa* (WBI) and *Elizabethkingia* sp. (3AS) was also tested after 4 and 24 h exposure. The *P. aeruginosa* strains were isolated from keratitis patients and the others from contact lens storage cases [13].

Table 2
Microbial Efficacy of cleadew System or Diluent against Clinical Isolates of Bacteria.

Microbiology test	Log10 kill (4 h)													
	S. maltophilia (ALC01)	*Achromobacter* sp. (S4)	*P. aeruginosa* (W9298)	*P. aeuriginosa* (WBI)	*Elizabethkingia* sp. (3AS)	*P. aeuriginosa* (CI3)	*P. aeuriginosa* (TU2)	*P. aeuriginosa* (TU4)	*S. marcescens* (UD3)	*S. marcescens* (TU9)	*S. epidermidis* (IT16)	*S. maltophilia* (UD14)	*A. faecalis* (CI1)	*A. xylosoxidans* (UD1)
Biocidal ("stand alone")	> 4	> 4	> 4	> 4	> 4	> 4.4	> 4.3	> 4.6	> 4.8	> 4.7	> 4.4	> 4.4	> 4.6	> 4.1
Biofilm	> 7	> 7	NT[a]	NT	NT	> 5.4	> 5.6	> 5.3	> 5.7	> 5.9	> 5.8	> 4.6	> 5.8	> 6.1
cleacare diluent only (4 h)	1.7	1.8	1.3	NT	1.9	NT	NT	NT	NT	NT	NT	NT	NT	NT
cleacare diluent only (24 h)	> 5	> 5	> 5	NT	> 5	NT	NT	NT	NT	NT	NT	NT	NT	NT
Biocidal using Japanese tap water (soft water) as diluent	NT	NT	NT	NT	NT	> 4.3	NT	NT	NT	> 4.5	NT	NT	NT	> 4.5
Biocidal using Japanese drinking water (hard water: cortex) as diluent	NT	NT	NT	NT	NT	> 4.5	NT	NT	NT	> 5	NT	NT	NT	> 4.7

[a] NT = not testedfigure.

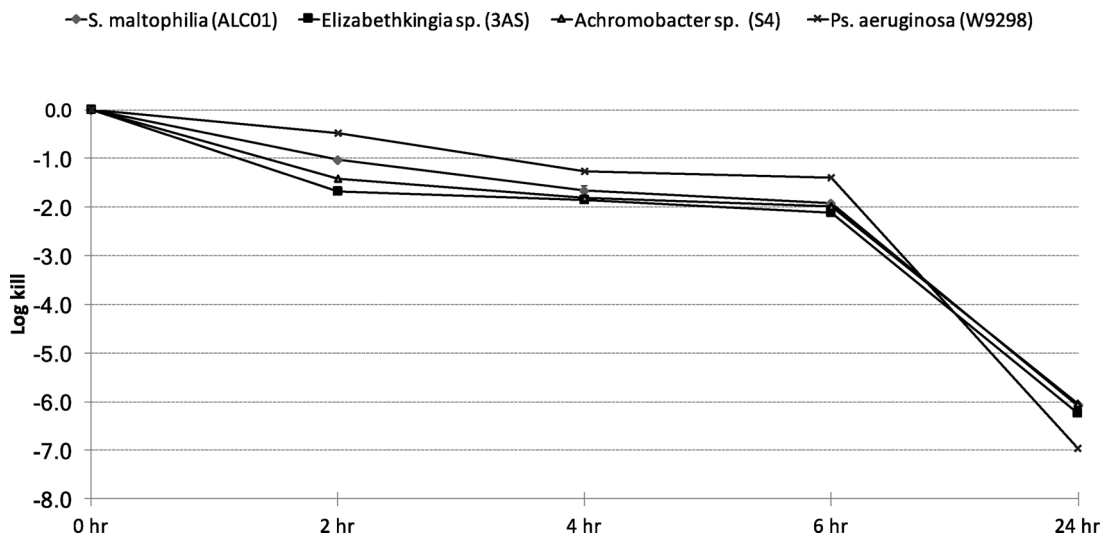

Fig. 1. Efficacy of cleadew diluent against bacterial strains isolated from contact lens storage cases.

3. Results

In the biocidal experiments using the ISO:14729 organisms or clinical bacterial strains, < 10 colony forming units (cfu) were recovered for all bacteria, yeast and mould tested after 4 h disinfection with and without the presence of organic soil. This equates to a > 4 $\log_{10}$ or > 99.99% kill (Table 1). Biocidal studies in which the challenged storages cases were kept for 14 days before sampling showed < 10 cfu, indicating that regrowth of the test organisms did not occur. In experiments in which the challenged cases were sampled after 20 min, < 10 cfu of bacteria were detected. Some surviving *Fusarium solani* were detected giving a 2.4 $\log_{10}$ reduction in viability. When 0.03% organic soil was used in the studies, no loss in biocidal efficacy was observed (Table 1). In the studies using tap or drinking water in place of the diluent (representing non-compliant use of the system), < 10 cfu were recovered for all bacteria, yeast and mould tested after 4 h disinfection.

In the regimen assay no viable organisms were recovered from the contact lenses after the manufacturer's recommended "rub and rinse" protocol for the system, with or without the presence 0.03% organic soil (Table 1). This included *Acanthamoeba* for which no adhering trophozoites or cysts could be observed on the lenses after the regimen treatment (Table 1).

In the biofilm studies using *S. maltophilia* or *Achromobacter* sp., the biofilm formed was composed of approximately 10^7–10^8 cfu. Following disinfection with the system, < 10 cfu were recovered with either organism (Table 2). Studies in which clinical bacteria were exposed to the diluent alone showed a > 1 $\log_{10}$ kill after 4 h and > 5 $\log_{10}$ by 24 h (Fig. 1).

For the *Acanthamoeba*, no viable trophozoites were detected after 4 h disinfection, equivalent to > 4 $\log_{10}$ kill. For the cyst stage, a 1.1 $\log_{10}$ kill was found with the NNA prepared cysts and 2.8 $\log_{10}$ kill with those produced using the Neff's encystment medium (Table 1). In the studies using tap or drinking water in place of the diluent, no viable trophozoites could be recovered after 4 h disinfection, indicating total kill of the challenge inoculum. Using the neutralized PI system, the solution did not induce *Acanthamoeba* encystment compared to the control solution which gave > 90%. Similarly, using the diluent alone resulted in no trophozoite encystment.

4. Discussion

Povidone iodine is a stable chemical complex of polyvinylpyrrolidone and iodine which is soluble in water. Developed in the 1950s, PI has been shown to exhibit a broad spectrum of antimicrobial activity including *Acanthamoeba* [14–19]. It is widely used as an antiseptic on wounds and the skin prior to surgical procedures including ocular surgery to reduce the risk of endophthalmitis [20,21]. As was shown here, PI is also effective against biofilm formation and biofilm containing organisms [14,16].

In this study it was demonstrated that the PI based contact lens care system was highly active against bacteria, fungi and *Acanthamoeba* in the planktonic and, in the use of *S. maltophilia* and *Achromobacter* sp., the biofilm state. As such, the system exceeds the stand-alone requirements of ISO 14729 [7]. Compared to MPS for contact lens disinfection, PI and H_2O_2 systems require neutralization before the lenses can be worn. This can allow surviving microbes to regrow during storage. It has been previously reported that regrowth of *Fusarium* spp. can occur over a 7–14 day period in these neutralized solutions [22]. The work was conducted with a previous version of the PI system that contained no preservative in the diluent solution. In this study, the latest version of the PI product was tested that contains 40 ppm H_2O_2 in the diluent solution. After adding the PI containing tablet, the ascorbic acid is released which neutralizes the PI turning the solution clear to indicate disinfection is complete which occurs after approximately 20 min. The H_2O_2 in the diluent also reacts with I^- formed in the neutralisation stage to generate additional I_2 to further enhance the biocidal activity of the system. Finally, the protease layer is released which acts to remove proteins from the lens surface. To ensure complete disinfection and removal of protein, the manufacuturer's recommended soaking time is 4 h. Although the neutralized system is deemed safe for the storage of lenses for up to 7 days, the findings of this study indicate that the diluent solution, containing 40 ppm H_2O_2 was bacteriacidal. This suggests that it may be advantageous to store the disinfected lenses in fresh diluent solution to ensure continued antimicrobial protection.

In addition, it has been reported that the interaction of iodine with hydrogen peroxide results in enhanced synergistic or additive inhibitory antimicrobial activity [23]. This resulted in no regrowth of bacteria or fungi up to 14 days. However, the manufacurer's recommendation is that the contact lenses be subject to full disinfection if stored for longer than 7 days. As reported previously, sensitivity in the eye to hydrogen peroxide occurs only at 100 ppm and above [6]. Although the PI system must only be used with the supplied diluent, it was shown that the PI tablet in tap water gave total kill of bacteria, fungi and *Acanthamoeba* trophozoites. Similarly, significant kill of bacteria and *Acanthamoeba* trophozoites was found after 20 min disinfection in the system, by which time the PI is fully neutralized. However, some surviving *F. solani* were detected at this time point but not

after 4 h. This suggests that the recovered conidia were viable but fatally affected and required a longer period of time to be killed.

The PI system was shown to be active against *Acanthamoeba* trophozoites and cysts as has been reported [18,24]. Unlike bacteria and fungi, no international standard exists for the testing of contact lens care solutions against *Acanthamoeba* [7]. In this present study, whilst ≥ 4 $\log_{10}$ kill was obtained against trophozoites, variation in cyst susceptibility was observed depending on method of preparation. Although a 2.8 $\log_{10}$ kill was found with cysts prepared using Neff's encystment medium, those prepared by starvation were markedly more resistant giving 1.1 $\log_{10}$ kill. This observation on the method of cyst production and susceptibility to disinfection has been published previously [10,25]. It is unknown in what state *Acanthamoeba* cysts occur is in the natural environment leading to acanthamoeba keratitis and further work on this topic is indicated.

Although the PI system must only be used with the supplied diluent, it was shown that the PI tablet in tap or drinking water gave equivalent kill of bacteria, fungi and *Acanthamoeba* trophozoites compared to when the diluent was used. Rinsing or storing of contact lenses in tap water is a known risk factor for acanthamoeba keratitis [3,26]. Therefore, this finding, along with that showing significant disinfection of bacteria, fungi and *Acanthamoeba* trophozoites after 20 min, indicates that the system can still provide effective disinfection even under highly non-compliant conditions of use.

Contact lens wear is a known risk factor for microbial keratitis and 90% of acanthamoeba keratitis cases occur in contact lens wearers [27–29]. Outbreaks of fusarium and acanthamoeba keratitis have occurred in the past and resulted in the recall of the associated MPS [30–32]. Compliance to recommended hygiene practices is fundamental to safe and comfortable contact lens use. The findings of this study indicate that the PI system is an effective contact lens disinfection system, even under non-compliant conditions and may help in reducing the incidence of microbial keratitis.

References

[1] F. Stapleton, L. Keay, K. Edwards, T. Naduvilath, J.K. Dart, G. Brian, et al., The incidence of contact lens-related microbial keratitis in Australia, Ophthalmology 115 (10) (2008) 1655–1662.

[2] N. Carnt, L. Keay, M. Willcox, V. Evans, F. Stapleton, Higher risk taking propensity of contact lens wearers is associated with less compliance, Cont Lens Anterior Eye 34 (5) (2011) 202–206.

[3] C.F. Radford, D.C. Minassian, J.K. Dart, Acanthamoeba keratitis in England and Wales: incidence, outcome, and risk factors, Br J Ophthalmol 86 (5) (2002) 536–542.

[4] K. Dalton, L.N. Subbaraman, R. Rogers, L. Jones, Physical properties of soft contact lens solutions, Optometry Vision Sci 86 (5) (2008) 122–128.

[5] R. Hughes, S. Kilvington, Comparison of hydrogen peroxide contact lens disinfection systems and solutions against *Acanthamoeba polyphaga*, Antimicrob Agents Chemother 45 (7) (2001) 2038–2043.

[6] B. Holden, A report card on hydrogen peroxide for contact lens disinfection, Clao J 16 (Suppl. 1) (1990) S61–4.

[7] I.S.O. International Organization for Standardization, ISO 14729: Ophthalmic optics – Contact lens care products – Microbiological requirements and test methods for products and regimens for hygienic management of contact lenses, International Organization for Standardization, Geneva, Switzerland, 2001.

[8] J. Behets, P. Declerck, Y. Delaedt, L. Verelst, F. Ollevier, Survey for the presence of specific free-living amoebae in cooling waters from Belgian power plants, Parasitol Res 100 (6) (2007) 1249–1256.

[9] M. Nikolic, S. Kilvington, Evaluation of the wasp (whitley automated spiral plater) and protocol colony counting system for antimicrobial efficacy testing of contact lens disinfectant solutions, Invest Ophthalmol Vis Sci 50 (5) (2009) 6363.

[10] S. Kilvington, A. Lam, Development of standardized methods for assessing biocidal efficacy of contact lens care solutions against Acanthamoeba trophozoites and cysts, Invest Ophthalmol Vis Sci 54 (7) (2013) 4527–4537.

[11] R.J. Neff, S.A. Ray, W.F. Benton, M. Wilborn, Induction of synchronous encystment in *Acanthamoeba* sp, Methods Cell Physiol 1 (1964) 55–83.

[12] S. Kilvington, W. Heaselgrave, J.M. Lally, K. Ambrus, H. Powell, Encystment of *Acanthamoeba* during incubation in multipurpose contact lens disinfectant solutions and experimental formulations, Eye Contact Lens 34 (3) (2008) 133–139.

[13] S. Kilvington, J. Shovlin, M. Nikolic, Identification and susceptibility to multipurpose disinfectant solutions of bacteria isolated from contact lens storage cases of patients with corneal infiltrative events, Cont Lens Anterior Eye 36 (Dec(6)) (2013) 294–298, http://dx.doi.org/10.1016/j.clae.2013.02.001 Epub 2013 Mar 1.

[14] M.J. Hoekstra, S.J. Westgate, S. Mueller, Povidone-iodine ointment demonstrates in vitro efficacy against biofilm formation, Int Wound J 14 (Feb(1)) (2017) 172–179, http://dx.doi.org/10.1111/iwj.12578 Epub 2016 Mar 10.

[15] P. Durani, D. Leaper, Povidone-iodine use in hand disinfection, skin preparation and antiseptic irrigation, Int Wound J 5 (3) (2008) 376–387.

[16] K.O. Oduwole, A.A. Glynn, D.C. Molony, D. Murray, S. Rowe, L.M. Holland, et al., Anti-biofilm activity of sub-inhibitory povidone-iodine concentrations against Staphylococcus epidermidis and Staphylococcus aureus, J Orthop Res 28 (9) (2010) 1252–1256.

[17] S. Gatti, C. Cevini, A. Bruno, G. Penso, P. Rama, M. Scaglia, In vitro effectiveness of povidone-iodine on Acanthamoeba isolates from human cornea, Antimicrob Agents Chemother 42 (9) (1998) 2232–2234.

[18] S. Kilvington, Antimicrobial efficacy of a povidone iodine (PI) and a one-step hydrogen peroxide contact lens disinfection system, Cont Lens Anterior Eye 27 (4) (2004) 209–212.

[19] T. Kobayashi, L. Gibbon, T. Mito, A. Shiraishi, T. Uno, Y. Ohashi, Efficacy of commercial soft contact lens disinfectant solutions against Acanthamoeba, Jpn J Ophthalmol 55 (5) (2011) 547–557.

[20] S.J. Isenberg, The ocular application of povidone-iodine, Community Eye Health 16 (46) (2003) 30–31.

[21] A. Grzybowski, P. Brona, Povidone-iodine is still a premium antiseptic measure in ocular surgery, Acta Ophthalmol 95 (May(3)) (2017) e253–e254, http://dx.doi.org/10.1111/aos.13144 Epub 2016 Jun 8.

[22] S. Kilvington, A. Lam, M. Nikolic, N. Brady, Resistance and Growth of Fusarium species in Contact Lens Disinfectant SolutionsOptometry and Vision Science *Fusarium* species in contact lens disinfectant solutions, Optom Vis Sci 90 (5) (2013) 430–438.

[23] E.I. Zubko, M.K. Zubko, Co-operative inhibitory effects of hydrogen peroxide and iodine against bacterial and yeast species, BMC Res Notes 6 (2013) 272.

[24] T. Kobayashi, L. Gibbon, T. Mito, A. Shiraishi, T. Uno, Y. Ohashi, Efficacy of commercial soft contact lens disinfectant solutions against *Acanthamoeba*, Jpn J Ophthalmol 55 (5) (2011) 547–557.

[25] S. Kilvington, C. Anger, A comparison of cyst age and assay method of the efficacy of contact lens disinfectants against Acanthamoeba, Br J Ophthalmol 85 (3) (2001) 336–340.

[26] S. Kilvington, T. Gray, J. Dart, N. Morlet, J.R. Beeching, D.G. Frazer, et al., Acanthamoeba keratitis: the role of domestic tap water contamination in the United Kingdom, Invest Ophthalmol Vis Sci 45 (1) (2004) 165–169.

[27] J.K. Dart, C.F. Radford, D. Minassian, S. Verma, F. Stapleton, Risk factors for microbial keratitis with contemporary contact lenses: a case-control study, Ophthalmology 115 (10) (2008) 1647–1654 1654 e1-3.

[28] F. Stapleton, N. Carnt, Contact lens-related microbial keratitis: how have epidemiology and genetics helped us with pathogenesis and prophylaxis, Eye 26 (2) (2012) 185–193.

[29] B.A. Holden, D.F. Sweeney, P.R. Sankaridurg, N. Carnt, K. Edwards, S. Stretton, et al., Microbial keratitis and vision loss with contact lenses, Eye Contact Lens 29 (Suppl. 1) (2003) S131–S134 discussion S143-4, S192-4.

[30] S.K. Ma, K. So, P.H. Chung, H.F. Tsang, S.K. Chuang, A multi-country outbreak of fungal keratitis associated with a brand of contact lens solution: the Hong Kong experience, Int J Infect Dis 13 (4) (2009) 443–448.

[31] W.B. Khor, T. Aung, S.M. Saw, T.Y. Wong, P.A. Tambyah, A.L. Tan, et al., An outbreak of Fusarium keratitis associated with contact lens wear in Singapore, JAMA 295 (24) (2006) 2867–2873.

[32] C.E. Joslin, E.Y. Tu, M.E. Shoff, G.C. Booton, P.A. Fuerst, T.T. McMahon, et al., The association of contact lens solution use and Acanthamoeba keratitis, Am J Ophthalmol 144 (2) (2007) 169–180.